Assessment of a molecular tool (CE-SSCP) to study balance of caecal flora of SPF piglet's groups

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Abstract

The aims of this study were to test the ability of CE-SSCP in describing the variability of the digestive contents flora of SPF (Specified Pathogenic Free) pigs from our experimental husbandry and to reach, by mixing individual samples, the concept of a digestive flora's profile characteristic of a batch of pigs. The faeces of six SPF sows were sampled and extracted DNA were mixed to constitute more and more composite samples. In addition, the caecal contents of 12 SPF piglets, issued from a single sow, were collected after slaughter at 28, 56 and 84 days postpartum and individually or after pooling tested. The DNA of each sample was extracted using the QIAamp DNA Stool Minikit. The PCR for amplifying the rDNA 16S V3 region was carried out on the individual DNAs and the mixed DNAs corresponding to each date of slaughtering. Then the PCR products were analysed by CE-SSCP. The reproducibility of the method has been tested first, and then the analysis of the profiles obtained from the faeces of the swine was conducted. It described a within-individual variability of about 40% when comparing SPF sows. When we mixed samples, variability between the profiles decreases with increasing number of faeces constituting the pool of DNA. Concerning the piglets, for each date of sampling, the pool of caecal contents DNA defined a characteristic group profile. Moreover, this profile varied with the age of the piglets. These results confirmed the use of CE SSCP as a tool for the description of the of digestive flora balances and their evolution at the batch level. This will be of particular interest in both animal health or food hygiene contexts associated with digestive flora perturbation.

Introduction

There are many issues regarding the composition, structure and stability of the digestive ecosystem. Until recently, only bacteriological techniques allowed us to describe the intestinal flora in a group of pigs reared and fed equivalently. The study of the intestinal microflora from a fecal matrix using classic bacteriological culture techniques thus revealed a wide microbial diversity (Moore et al., 1987). The bacterial population in intestinal contents is estimated between 10^6 and 10^11 bacteria per gram of contents. However, a major limitation is related to the cultivability of the species studied. According to the results of 16S ribosomal DNA sequences analysis after PCR amplification on feces 80% to 90% of the bacteria present in feces cannot be cultivated because of their unknown metabolic requirements (Zoetendal et al., 1998). Simpson et al., 1999). CE-SSCP (Capillary Electrophoresis-Single Strand Conformation Polymorphism) which targets the 16S-rDNA V3 region, allows detection of bacteria whatever their physiological and metabolic status. Applied on digestive content analysis, it could enlarge the field of investigation compared to conventional bacteriological techniques concerning complex microbial ecosystems. (Dabert et al., 2005).

Maintaining the balance in the digestive flora appeared as a factor of prevention as well for product hygiene (e.g. Salmonella excretion, Beloeil et al., 2004) as in animal health (e.g; weaning pigs transition Simpson et al., 2000) perspectives. However, before determining favorable or unfavorable balances, the within-individual variability of animals needs to be assessed (Simpson et al., 2000), and then its influence should be minimized. Mixing the feces of animals from the same group and use of the CE-SSCP technique could then make it possible to characterize the balance of the digestive ecosystem of groups of pigs.
The objective of this study is to obtain a description of microbial composition of high-sanitary status pigs feces using CE-SSCP, then to measure the variability of SPF pig digestive contents, and to determine using a mixing sample approach to limit this variability, the ability to reach the notion of flora profiles of pig batches.

Materials and methods

The feces of six SPF sows (identified from number one to six) of different ages, parity numbers and fed with different diets were sampled in order to test the usefulness of the CE-SSCP method in describing flora (repeatability, within-individual and inter-group variability were checked).

Twelve SPF piglets from AFSSA's experimental piggeries, born from the same sow were also used in this study in order to determine the profiles of their cecal flora and to investigate their evolution over time. The piglets were randomized to three groups of 4 piglets on weaning day and slaughtered the same day for the first group to collect cecal contents. Cecal content were identified with number 1, 2, 3 and 4. The second group (pigs identified 5, 6, 7 and 8) and third (pig number 9, 10, 11 and 12) were sacrificed at 28 and 56 days post-weaning, respectively. A starter feed was given to the piglets from the first week of age in the maternity until two weeks after weaning (21% protein). The transition feed was provided at 42 days of age (for a transition period of about 2 weeks, 18% protein). The grower feed started from 56 days of age until the end of the trial (16% protein).

DNA extraction from the samples was performed using a QIAamp DNA Stool Minikit (Qiagen) (McOrist et al., 2002). DNAs in the feces of sows 1, 2, 3, 4, 5, 6 were extracted and mixed as follows: DNAs 1-2-3, 4-5-6, 1-2-3-4, 1-2-3-4-5 and 1-2-3-4-5-6. DNAs in piglet cecal contents were extracted and the following mixs were carried out: 1-2, 3-4, 1-2-3-4, 5-6, 7-8, 5-6-7-8, 9-10, 11-12, 9-10-11-12. Gene amplification of DNAs and DNA pools was performed according to the methods described by Tanguy et al. (2007). CE-SSCP consisted in the migration of DNA single strands into the 50-cm capillaries of the four-capillary AbiPrism Genetic Analyser 3100 Avent sequencer (AppliedBiosystem, France). Profiles were classified via dendrograms built using the BioNumerics software (Applied-maths, France) and the Jaccard coefficient for similarity determination according to Tanguy et al (2007).

Results

SSCP profiles were obtained from sows fecal samples. Each profile included 50 to 60 peaks (Figure 1). The total reproducibility of the method was observed after comparison of several profiles of the same sample subjected to different protocols. The peaks number and position were identical. Results showed by CE-SSCP analysis were reproducible as early as DNA extraction and allowed to generate flora profiles from sows composite fecal samples.

Figure n°1: Profile representations of two DNA extractions were carried out in parallel from the same pool of feces of 6 sows and profiles of two PCR of an individual DNA extraction from the feces of the 6 sows and the DNAs mixed after extraction.
The SPF sows included in this study did not have any pathology, had not received any medication and were given two diets based on their physiological stage (gestation or lactation). Analysis of the profiles obtained from these sows feces showed low similarity of individual profiles ranging between 59% and 75%.

DNA mix samples revealed an increase in the number of individuals similar in pools 1-2-3 and 4-5-6. Thus, profile similarity, proportional to the increase in the number of individuals included in the pool. Thus, profile was 81%, while it reached, and even exceeded 90% for mix 1-2-3-4, 1-2-3-4-5 and 1-2-3-4-5-6 (Figure 2).

Figure 2: profile similarities of individuals or mix of 6 sows feces (Jaccard coefficient and UPGMA average)

The analysis of the profiles obtained from piglet cecal flora, presented in Figure 3, described three distinct clusters grouping the piglet according to their age. Inside each cluster, the within-individual variability is low. The minimal similarity between individuals being at least 68.8%, 81.3% and 88.6% for the 28 days aged piglets (in groups 1 to 4), 56 d aged (5 to 8) and 82 d aged (9 to 12), respectively (Figure 3). In our confined piggeries the similarities of the caecal profile in a pig batch increase with the time.

In these conditions, we wanted to precise the place of the mix profiles in the dendrogram. This analysis confirmed that each composite samples integrated the cluster characteristic of the piglet group at each slaughter date. Profiles 1-2, 3-4 and 1-2-3-4 are thus representative of the group associating piglets 1 to 4 slaughtered at d28, profiles 5-6, 7-8 and 5-6-7-8 are characteristic of the piglets in group 5 to 8 slaughtered at d56 and profiles 9-10, 11-12 and 9-10-11-12 are representative of the profiles of individuals 9 to 12 slaughtered at d84. In addition, the similarity evolution, according to piglet age observed over time, is reinforced when the analysis is carried out on pools. Group 1-2-3-4 similarity is thus evaluated at 58% compared to groups 5-6-7-8 and 9-10-11-12, respectively, a 64% similarity being found between groups 5-6-7-8 and 9-10-11-12. These results showed that we can analyse the evolution of the pig group caecal flora profiles with composite samples inside a batch.
**Figure 3:** Analyse of profile similarities of individuals or pools of 12 piglets cecal floras using Jaccard coefficient and UPGMA average (slaughter date: 28 days (piglets 1, 2, 3, 4), 56 days (piglets 5, 6, 7, 8) and 84 days (piglets 9, 10, 11, 12))

**Discussion**

The CE-SSCP method, used for studying pig digestive microflora, provides reproducible profile even for separate sample DNA extractions. However, we observed that if we decided to pool samples within a study, the mix must be operated in a standardized way. For example it is to be decided once for all whether to pool samples, before or after DNA extractions. The sow digestive flora profile analysis revealed within-individual variability between healthy high-sanitary-status sows (59%-75% similarity). Two sows were changed diet ten days prior to the study (gestation feed replaced by a lactation feed), but the change did not significantly affect their individual flora profiles. Mixing DNAs made it possible to ignore the variability between sows, and showed that it was possible to tend toward a digestive flora profile characteristic of a pig or sow herd (similarity higher than 90% with a feces mixture from at least 4 sows). The analysis of all piglet cecal content profiles described a distribution in three clusters according to piglet age. Evolution with time of gastrointestinal microbiota contents in piglet was previously described using molecular approach (Simpson et al 2000). Analysis with CE-SSCP (at least 50 peaks) per profile would allow describing smaller variation than dgge (maximum 35 bands) (Loisel et al. 2006). Profile similarity (68.8%) within the weaning piglet cluster (d28) further increased with time to reach 90% similarity. The diet of pre-weaned piglets does not exclusively consist of the sow's milk, but also of starter feed to which piglets have free access. The diet, and hence the development of the flora in piglets, could thus be determined according to the piglets' interest in the starter feed, and could therefore account for the lower similarity between the profiles of the animals at this age. The mix profiles of the 3 groups integrate within the clusters of piglets treated individually; they are therefore representative of the piglets in these clusters. Thus, the results show that cecal flora profiles have a strong within-individual similarity between piglets of the same age which evolves identically with piglet age within the group.

**Conclusion**

In conclusion, capillary SSCP is a method that allows to describe digestive contents of sows and piglets qualify their variability. Using this method we described the evolution of piglet groups profiles over time (from the post-weaning through grower phases). The strategy of mixing intestinal contents in a way to enforce similarity between individuals digestive flora was validated. It provides a tool for studying digestive flora balances and their breakdown. This will be of particular interest in both animal health or food hygiene contexts associated with digestive flora perturbation.

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**Bibliography**


